CSF Procedure Manual November 2005

Classical Swine Fever (CSF) Surveillance Procedure Manual

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The Enhanced Classical Swine Surveillance Program
October 1, 2005

The enhanced Classical Swine Fever (CSF) surveillance program outlined in this manual is the result of cooperative efforts by several Veterinary Services sections. As early as 2002 several parties under the guidance of Veterinary Services were developing plans for the emerging National Animal Health Laboratory Network (NAHLN) to provide the nation with increased diagnostic testing capacity for foreign animal disease agents of interest, including CSF. Meanwhile, research conducted at ARS facilities at Plum Island in cooperation with the Foreign Animal Disease Diagnostic Laboratory (FADDL) branch of the National Veterinary Services Laboratories (NVSL) resulted in the development and validation of a real-time Polymerase Chain Reaction (rtPCR) diagnostic test for CSF which was completed in 2005.

The newly-developed National Surveillance Unit (NSU) was charged with developing a risk-based surveillance plan for CSF. Dr. Eric Bush led the design of the enhanced surveillance plan, which is included in this manual to provide the rationale for the sampling protocols. Incorporation of the rtPCR test for rapid detection of viral DNA into the expanding NAHLN is a major component of the plan.

When the Veterinary Services (VS) Management Team identified funding for the NAHLN Laboratory Network early in FY 2005 and for implementation of the revised CSF surveillance plan mid-year, staff from the National Center for Animal Health Programs (NCAHP), in cooperation with NSU, NVSL, and Regional staff, began developing the agreements and budget information necessary to implement parts of the program (Cooperative Agreements) by September 30, 2005.

Because funding to allow implementation of this program was an uncertainty, and certainly a pleasant surprise, we have not had the time necessary for a smooth implementation of the CSF surveillance program. This manual is an initial attempt to rectify this situation. It is intentionally designed in a binder format with dated sections to allow easily replacements, additions, and revisions.

Because of the many new procedures, relationships, and policies being initiated in this program, we can foresee that changes will need to be made in the protocols and procedures as we together build a cost-effective surveillance program. Please keep us all informed as to what is working (and what is not) as we move forward in this cooperative effort. Pertinent contact information is listed at the end of each section of the manual for your reference.

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I. Introduction

A. Purpose

This document describes the guidelines and procedures for the national surveillance program for classical swine fever (CSF) beginning in November 2005. The goal of the program is to enhance surveillance for the rapid detection of CSF virus introduced into US swine. Samples will be collected in the 18 high risk states, which are AZ, CA, FL, GA, HI, IL, IN, IA, KS, MN, NE, NJ, NM, NY, NC, OK, TX, and WA.

The purpose of this document is to clarify:

- The objective of the overall surveillance program
- When to refer a highly suspicious for CSF animal to the Area Veterinarian-In-Charge (AVIC) for a possible Foreign Animal Disease (FAD) investigation
- When and how to sample targeted high risk swine
- What information to record about the sample
- How to ship the sample
- Where to ship the sample
- Communication protocols

B. CSF description

Classical Swine Fever (CSF) is a highly contagious viral septicemia affecting only swine. Also known as Hog Cholera, it has been eradicated from many developed nations with extensive swine production but is still endemic in much of the world. Outbreaks in countries free of CSF can have a severe impact on producers due to high swine mortality, the curtailment on exportation of swine and pork products, and from costs incurred to control and eradicate the disease.

1. **Etiologic Agent**. The etiological agent of CSF is a small enveloped RNA virus of the family Flaviviridae and genus Pestivirus, which also includes the Bovine Viral Diarrhea (BVD) virus and Border Disease (BD) virus. CSF virus is stable in cool, moist, protein-rich environments such as pork and pork products and can survive in cured or smoked pork for up to188 days and over 4 years for frozen pork.

2. **Clinical signs**. The clinical manifestation of CSF depends primarily on the viral strain, as field strains vary widely in their virulence. Host characteristics also play a role, particularly the age of the host (more severe disease in young pigs), immune status, nutritional condition, and breed. Generally though, CSF manifests either as an acute, chronic, or late-onset infection of swine.

   Acute infection is the more ‘classical’ presentation of CSF and is usually seen in piglets 12 weeks old or less. Pathological lesions are most commonly found in tonsil, lymph nodes, spleen and kidneys and reflect those of a septicemic disorder with multiple hemorrhages of various sizes. Infarcts of the spleen are considered pathognomonic for CSF when present. Antibodies become detectable 2-3 weeks post

infection, with a practical minimum of 18 days. Several domestic disease conditions produce a similar clinical picture.

Chronic infection consists of three phases and is always fatal though animals may survive 2-3 months before dying. Antibodies may only be detectable temporarily during the first month of infection but then disappear and can not be detected.

"Late onset" infection occurs when pregnant swine are infected with CSF virus. Infections prior to day 50 of gestation result in abortions, stillbirths, mummies, or birth of deformed piglets. The clinical signs in sows are usually mild, nonspecific and not indicative of CSF.

For sows infected about 50-70 days of gestation, piglets will be born persistently viremic (similar to BVD viral infection in calves) and may be clinically normal for months or may exhibit congenital tremors from birth. Eventually, at 2-11 months of age, pigs will begin to waste and become unthrifty. Persistently infected pigs shed virus constantly until they die!

3. **Epidemiology**. Movement of normal looking infected pigs is the most frequent method of transmitting CSF virus. Other important sources include infected feral swine and contaminated pork and pork products. Virus can be shed in any bodily secretion including semen. The most frequent route of infection is oronasal. Important mechanical vectors for introduction of virus into a herd include transport vehicles and people.

   The rate of transmission between swine within a breeding herd is slower than the transmission rate between weaned pigs. Therefore, CSF may be present in populations of breeding stock for quite some time before it is noticed. An infected herd will be detected sooner if the infection starts in the nursery or finisher section than when the infection starts among the breeding stock.

   In experimentally infected swine the incubation period averages 7-10 days (range of 3-15 days). Under field conditions, the incubation period is approximately 2-4 weeks. The expected morbidity rates are 33-45% of pigs at risk. Between 15-30% of cases can be expected to die.

4. **Economic impact**. The economic impact of CSF can arise from excessive mortality, infertility, and other deleterious health effects at the herd level. A severe economic consequence of an incursion of CSF into the US is the immediate halt to exports. The US pork industry currently exports over 12% of its annual production with a value of more than $1.5 billion. The US is the world's second largest exporter of pork.

   A significant impact is the cost of disease control and eradication. US costs for the eradication of CSF totaled more than $140 million in 1978. This would be more than $540 million in 1999 dollars. Direct cost of The Netherlands control program for CSF in 1983-85 was $93 million compared to the 1997-98 Netherlands outbreak in which costs associated with the slaughter of infected and exposed swine, production

prohibitions, welfare slaughter, movement restrictions, and effects on allied industries exceeded $2 billion.

C. Surveillance Plan Overview

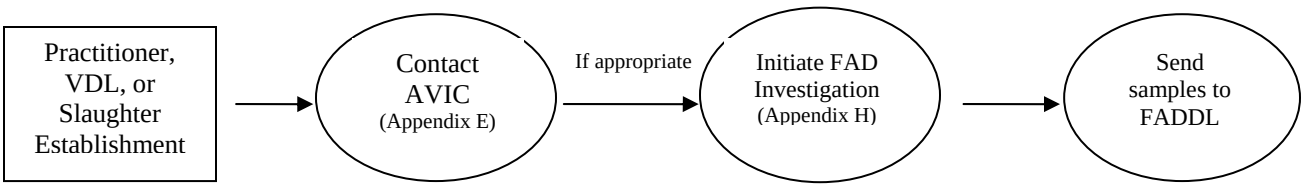
The Animal and Plant Health Inspection Service (APHIS), in cooperation with the National Animal Health Laboratory Network (NAHLN), has begun an enhanced national CSF surveillance plan to detect more rapidly any introduction of CSF virus into US swine. The goal is to test targeted swine populations in high risk states. The swine populations targeted for surveillance include: 1) swine highly suspicious for CSF, 2) sick pigs submitted to a veterinary diagnostic laboratory, 3) pigs condemned at slaughter by the Food Safety and Inspection Service (FSIS), and 4) feral swine taken by Wildlife Services (WS).

For questions about the CSF Surveillance Plan, contact Eric Bush (970-494-7260 or Eric.J.Bush@aphis.usda.gov).

1. Swine Highly Suspicious for CSF

Swine fit this category if the clinical signs of CSF are observed as described in the case definition (Appendix A). The majority of these observations are made on the farm but may also be made at diagnostic laboratories or slaughter establishments. **All swine that fit this category should be referred to the AVIC in your state** (Appendix C). The AVIC or their designee will determine if a full FAD investigation is warranted and assign a Foreign Animal Disease Diagnostician (FADD) to collect appropriate samples. **Note**: The procedures for collecting a sample as part of an FAD investigation are different from the regular surveillance sampling procedures. See Appendix G for a copy of VS Memorandum 580.4 that provides the guidance for conducting FAD investigations.

For questions regarding sampling and submitting samples for FAD investigation, contact Samia Metwally (631-323-3322 or Samia.A.Metwally@aphis.usda.gov).



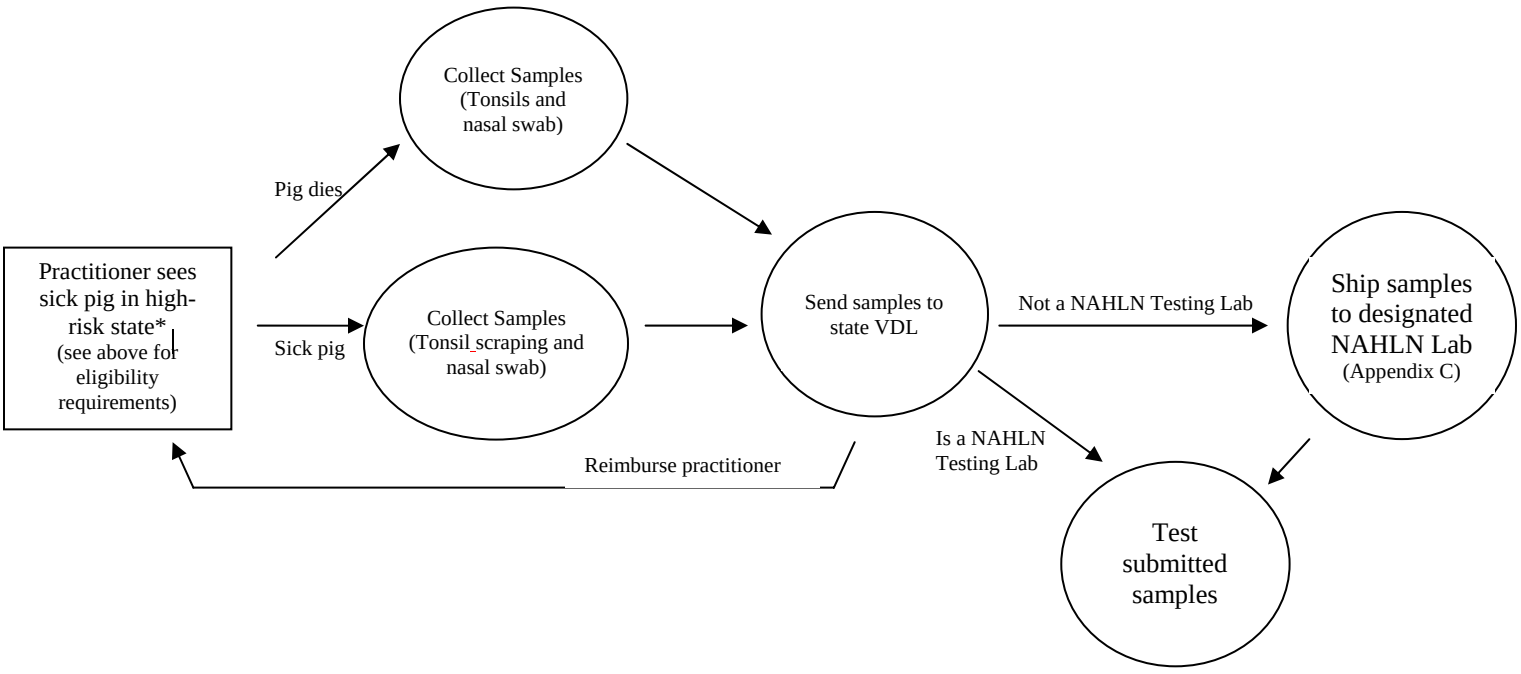
2. Sick pigs submitted to a veterinary diagnostic laboratory

Any swine accession received by a diagnostic laboratory in a high risk state (except Iowa and Minnesota) is eligible for testing at a CSF-approved NAHLN laboratory.

Because of the volume of swine accessions at the Iowa and Minnesota veterinary diagnostic laboratories, eligible specimens from these two states are limited to only those cases with any of the following:

- Dramatic acute septicemias
- Abortions, particularly with congenital deformities
- Dermatitis or Nephritis (PDNS is a rule out)
- Undiagnosed CNS cases (especially congenital tremors & nonsuppurative encephalitis)
- Other undefined cases that the pathologist wishes to submit

Questions regarding submitting specimens to a VDL can be directed to the designated NAHLN laboratory or contact the appropriate USDA-APHIS-VS Regional Epidemiologist: Mark Schoenbaum (Western region, 970-494-7314 or Mark.A.Schoenbaum@aphis.usda.gov) or Donald Rush (Eastern region, 919-855-7230 or Donald.M.Rush@aphis.usda.gov).

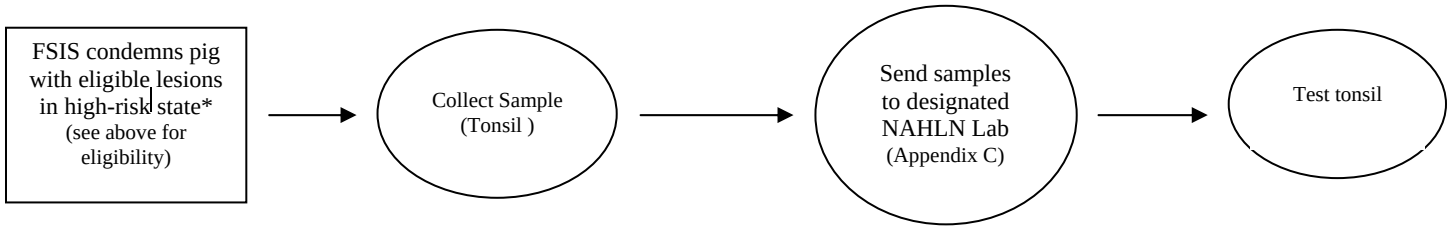


*High Risk states are: AZ, CA, FL, GA, HI, IL, IN, IA, KS, MN, NE, NJ, NM, NY, NC, OK, TX, and WA

3. Pigs condemned at slaughter by FSIS

Pigs eligible for sampling are those condemned for erysipelas or septicemia. Specimens will be obtained from swine slaughtered at establishments in high risk states (24 plants that slaughter over 500,000 market swine a year plus an additional 10 plants). The selected slaughter establishments cover over 95% of market swine slaughtered in high risk states and over 75% of all US slaughter.

Questions regarding submitting specimens to a VDL can be directed to the appropriate USDA-APHIS-VS Regional Epidemiologist: Mark Schoenbaum (Western region, 970-494-7314 or Mark.A.Schoenbaum@aphis.usda.gov) or Donald Rush (Eastern region, 919-855-7230 or Donald.M.Rush@aphis.usda.gov).

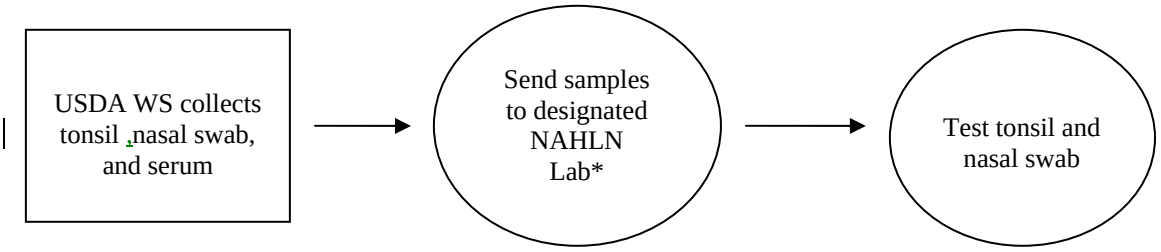


*High Risk states are: AZ, CA, FL, GA, HI, IL, IN, IA, KS, MN, NE, NJ, NM, NY, NC, OK, TX, and WA

4. Feral swine taken by WS

Free-roaming feral swine in states where WS biologists conduct feral swine damage management will be sampled periodically. These states include CA, FL, GA, HI, MO, NC, OK, OR, SC, TX, and PR.

Any questions regarding sampling from feral swine can be directed to Seth Swafford (301-734-3570 or Seth.Swafford@aphis.usda.gov).



* Serum samples are not currently being tested for CSF at NAHLN labs. Serum samples should be shipped to FADDL.

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II. Detailed sampling procedures

A. Tools needed

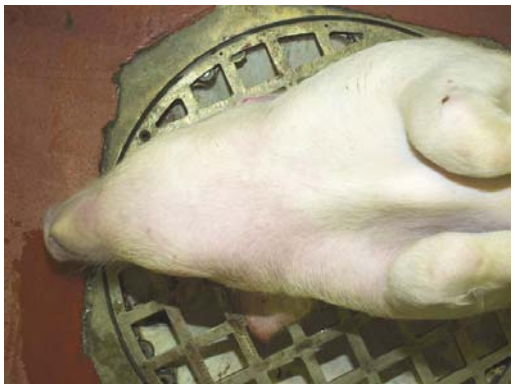
- For removing tonsils:
  - Knife and scissors
  - Forceps
  - Screw-top plastic tubes
- For tonsil scraping:
  - Sterile long-handled spoon
  - Speculum
  - Dacron swab
  - Sample tube containing 1.5 ml of DMEM with antibiotics
- For nasal swab:
  - Dacron swab
  - Sample tube containing 1.5 ml of DMEM with antibiotics
- Fine point permanent marker
- Ball-point pen
- Pan or bucket for disinfecting instruments and rinsing gloved hands
- Bleach (disinfectant)
- Paper towels
- Trash bags
- Supply of CSF mailers (including frozen cold packs)

B. Steps in collecting specimens

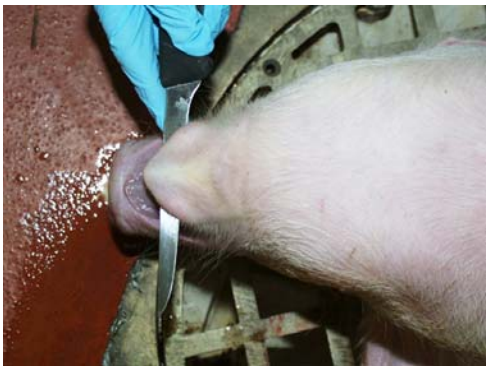
The objective would be to collect samples for virus isolation or other antigen based assay systems. The tonsils, a tonsil scraping or a nasal swab should be taken for surveillance testing.

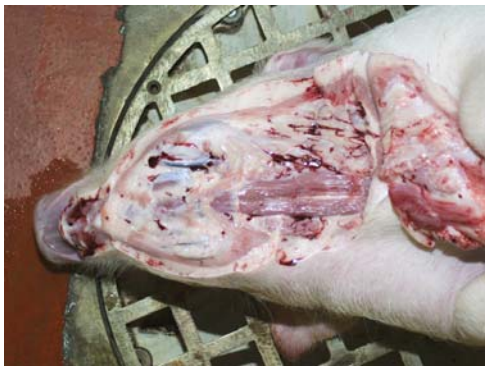
Removing tonsils (Dead pigs)

1. Lay the pig in dorsal recumbency.

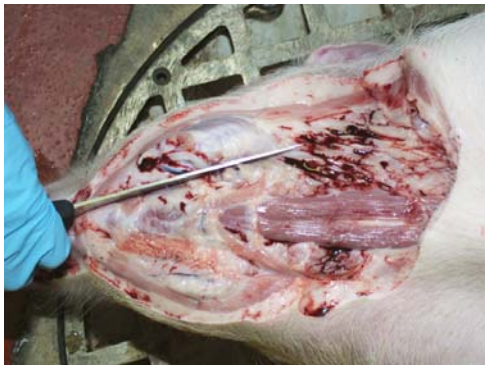


2. Beginning near the chin, use a knife to reflect the skin caudally to expose underlying tissues in the intermandibular and proximal cervical regions.

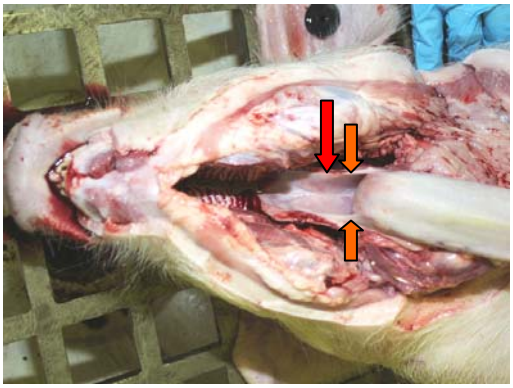




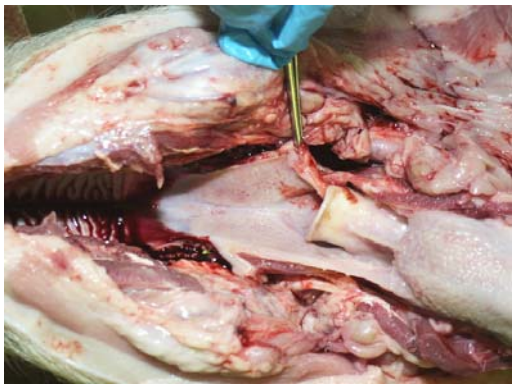
3. Incise soft tissue along the medial aspect of each mandible. Extend proximally to the mandibular symphysis on each side in order to free the attachments of the tongue.



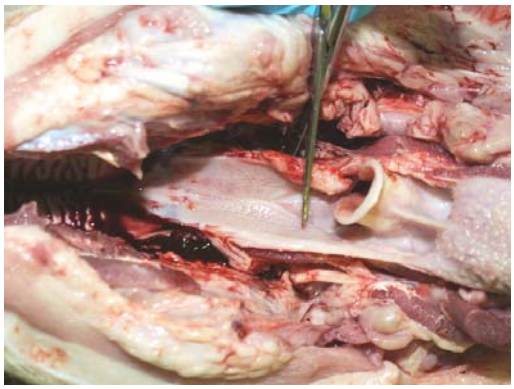
4. After freeing up the proximal attachments of the tongue, reflect the tip of the tongue caudally to expose the hard and soft palate. The palatine tonsil, a flat bi-lobed structure with a prominent medial septum, is located caudal to the soft palate (red arrow). Cut the lateral attachments that restrict further retraction of the tongue (orange arrows).



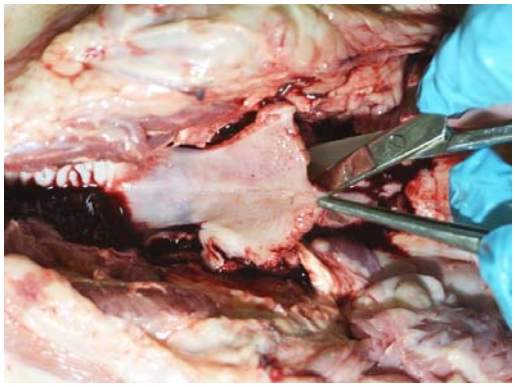
5. Reflect the tongue further to expose the tonsil and epiglottis. Note the dimpled appearance of the flattened tonsil, due to invaginations of the epithelium to form crypts.



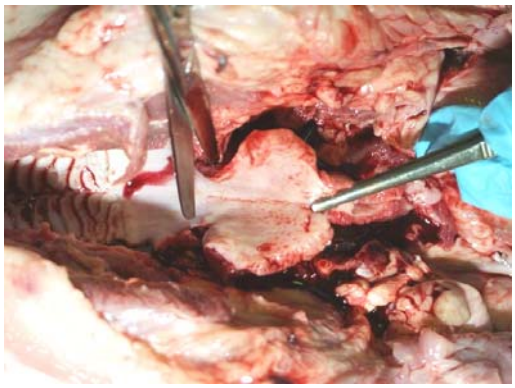
6. Use scissors to separate the tonsil from laryngeal structures caudal to it.



7. Grasp the caudal aspect of the tonsil with forceps and use scissors to cut the deep attachments of the tonsil.



8. Cut the proximal attachments of the tonsil to the soft palate. The tonsil is now freely excised and can be removed.



9. Place tonsils in sample tube.

Tonsil Scraping (Sick pigs)

1. Prop the mouth open using a speculum and place the bowl of the sterile spoon past the hard palette down into the upper throat. A long-handled spoon facilitates collection in market age or larger swine with longer palettes. The tonsil is just past the hard palette and is recognized by the pitted appearance of its surface.

2. Gently scrape the bowl of the spoon over the surface of the tonsils in a back-to-front motion several times. This will cause the tonsil to exude a mucosal excretion from the crypts.

3. On the third or fourth pass over the tonsil, the bowl of the spoon will collect a significant amount of sample, sometimes as much as 1-2 ml. **Do not scrape too hard, as drawing blood is not desired.**

4. Remove the spoon from the mouth, taking care to avoid dragging the spoonful of sample across the hard palate.

5. Remove the sample from the spoon using a Dacron swab and place in the sample tube containing 1.5 ml of DMEM with antibiotics.

Nasal Swab (Sick and Dead pigs)

1. The pig should be properly restrained with the head positioned upward to allow easy access to the nasal cavity. Anesthesia is not needed.

2. Insert a sterile Dacron swab into the nasal cavity and gently swab the surface of the nasal mucosa with a circular and back and forth motion to cover as much as possible of the nasal mucosal surface. Avoid touching the skin as you enter the nasal cavity.

3. The swab will collect nasal mucosal secretions and surface epithelium. It is important not to scrape too hard, as drawing blood is not desired.

4. Remove the Dacron swab from one nostril and repeat the same procedure in the other nostril.

5. Place the Dacron swab with sample in a tube containing 1.5 ml of DMEM medium. Stir the nasal swab into the medium so that the sample is washed out from the swab into the medium.

Proper labeling of samples

- Label each tube with a smear/waterproof pen. Include on each label:
  - Sample number,
  - Type of specimen in tube (tonsil or scraping, nasal swab),
  - Barcode ID label
    - Bar codes are printed in sets of 8 individual labels. Each sample should receive a different bar code, even if several samples are collected from the same animal.
    - Bar codes should be used as follows:
      - 1 label on each sample tube – be sure to place bar-code **lengthwise** along the tube.
      - 1 label on the submission form
      - Any labels that are not used should be destroyed

- Place the samples in a cooler and/or on cold packs. **Do not freeze specimens**.
- Properly dispose of non-submitted tissues and/or carcass.

Any questions regarding sampling techniques can be directed to the program managers at USDA-APHIS-VS-NAHPS, David Pyburn (515-284-4122 or David.G.Pyburn@aphis.usda.gov) or John Korslund (301-734-5914 or John.A.Korslund@aphis.usda.gov), or to FADDL (631-323-3256).

III. Submitting specimens to a NAHLN laboratory

Packing and shipping the specimens

1. **Packaging material** (supplied by NVSL)

   a. CSF Sample Collection Kits

   - Conical tubes, 50 ml (for tonsils)
   - Sterile Dacron swabs
   - Approved shipping box (TC-34)
   - Ice packs (2)
   - Absorbent material
   - 40-section box
   - Secondary container (STP-740 and STP-741)
   - Bar codes
   - UN/Diagnostic Specimens label

   b. CSF Media Kits

   (for tonsil scraping and nasal swabs)

   - Conical tubes, 15 ml, with 1.5 ml DMEM (20/box)
   - 40-section box (2)

2. **Packaging and shipping**

   - Place labeled sample tubes into the clear bio-hazard bag (STP-741) with absorbent and seal.

   - Place this bag into white bio-hazard bag (STP-740) and seal

- Place the white bag into the shipping box.
- Place frozen ice packs on top of the bag.

- Place completed USDA CSF Surveillance Submission Form on top of inner Styrofoam lid.

- Seal box
- Place address shipping label on the box (supplied by local Federal veterinarian), addressed to designated laboratory conducting CSF testing for this collection site.
- Place the other required shipping labels on the box.

- Ship by overnight delivery with the Federal contract service.
- **If shipping on a Friday, be sure to mark/label box for Saturday delivery.**

NVSL supplies a certified shipping box and all supplies needed for shipping as CSF kits. To request additional CSF kits, fax a request to: **515-663-7378**. If you need further assistance with shipping, you may contact the shipping department at:

National Veterinary Services Laboratories
1800 Dayton Avenue
Ames, IA 50010
Ph: (515) 663-7530
Fax: (515) 663-7378

A. Designated NAHLN Laboratory

Ship specimens via the overnight contract delivery service only to the designated NAHLN laboratory. See Appendix D to identify the designated NAHLN laboratory which is determined by the sampling site.

B. Proper communication of submitting specimens

It is essential to have secure and reliable communication among the individuals responsible for sample collection at collection locations, establishments' management, and NVSL or designated laboratories.

The submitter must:

- Accurately record all relevant information on the USDA CSF Surveillance Submission Form (Appendix D). Enter this information via the web-based forms unless such electronic entry is impossible. Print a copy of the completed CSF Surveillance Submission Form.

- Prepare three (3) copies of the completed CSF Surveillance Submission Form:
  1. One to accompany the samples shipped to the designated laboratory,
  2. One to be kept on-file by the submitter,
  3. One to be sent to and kept on-file at the VS Area Office

- Notify the appropriate laboratory (Appendix D) of incoming samples via facsimile, telephone, e-mail, or any other approved electronic method. The information to be communicated includes:
  - The overnight contract delivery service tracking number,
  - The collection site name and address,
  - The unique Referral Number of the submission, and
  - The number of samples.

- Verify, via the overnight contract delivery service tracking system, that the submission has been delivered to the designated laboratory. If the sample does not arrive as expected, the sample submitter should work with the delivery service to determine the location and delivery status of the sample.

Any questions regarding submitting specimens to the designated NAHLN laboratory can be directed to the NAHLN coordinator, Barbara Martin (515-663-7731 or barbara.m.martin@aphis.usda.gov).